GRAPPA (Group for Research and Assessment of Psoriasis and Psoriatic Arthritis)
Pilot Research Grant, 2023 Academic Year

To Whom it May Concern:

Our grant proposal, titled "TRPM4 Function in Western Diet Induced Psoriasis via IL-23 Mediated Inflammation", accompanies this Cover Letter.

We are responding to the GRAPPA Pilot Research Grant in the category of **Basic Science**.

Dermatologist (Mentor, Professor, and Department Chair):
Samuel Hwang, M.D., Ph.D., University of California Davis

Medical Student (Mentee and Lab Researcher):
Omar Alzayat, B.A., University of California Davis

Thank you for your consideration.

Sincerely,

Omar Alzayat
UC Davis School of Medicine
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Sacramento, CA 95817
Cell Phone: 510-423-2043
Email: Oalzayat@ucdavis.edu

TRPM4 Function in Western Diet Induced Psoriasis via IL-23 Mediated Inflammation

Omar Alzayat (Mentor: Samuel Hwang, M.D., Ph.D.)
University of California Davis, Department of Dermatology
University of California Davis, School of Medicine

Summary and Hypothesis:

Transient receptor potential melastatin 4 (TRPM4) is a calcium-activated, monovalent cation channel that is expressed in many cells. Dr. Hwang (mentor) and colleagues have reported that two gain-of-function (GoF) mutations of TRPM4 are the cause of progressive symmetric erythrokeratodermia (PSEK), which shares clinical and histologic similarity to psoriasis (Fig. 1), particularly with respect to thickening of the epidermis[1]. We then generated mice that bear one of two known TRPM4 GoF mutations and have shown that these mice were more susceptible to psoriatic skin inflammation[3].

We believe that TRPM4 is involved in cellular functions that are key to psoriatic inflammation and hypothesize that TRPM4 is involved in cellular processes that regulate immune cell migration and proliferation, as well as keratinocyte function, which ultimately impacts the ability of skin to develop thickening and scaling. We will test this hypothesis by demonstrating differences between the biologic behavior of cells isolated from mice which were genetically modified to mimic one of the human TRPM4 mutations that cause PSEK vs. wildtype littermates. We will also determine if a recently described small molecule inhibitor of TRPM4, "Compound 5" (a halogenated anthranilic amide)[2], blocks psoriasis-like inflammation in mice when administered in vivo, thus raising the prospect for a new class of medication for psoriasis that target TRPM4.

Background and Rationale:

Based on using CRISPR/Cas9 technology, TRPM4I1029M mice were previously generated that have the equivalent mutation to one of the two genetic mutations found in human PSEK (equivalent to human TRPM4I1033M). In the absence of experimental stimulation, TRPM4-I1029M mice did not present with an observable phenotype. When treated with imiquimod (IMQ), which has frequently been used to trigger psoriatic inflammation, TRPM4I1029M mice were predisposed to more severe PsD than WT which was characterized by a substantially greater accumulation of CCR6-expressing γδ T cells in LN and higher mRNA levels of Il17a, generating increased skin inflammation[3] (Fig. 2A-B).

In TRPM4I1029M mice, dendritic cells showed enhanced migration and keratinocytes exhibited increased proliferation via an in vivo migration assay[3]. We speculate that dendritic cell (DC) migration and keratinocyte proliferation would increase via TRPM4 GoF, as both have been shown

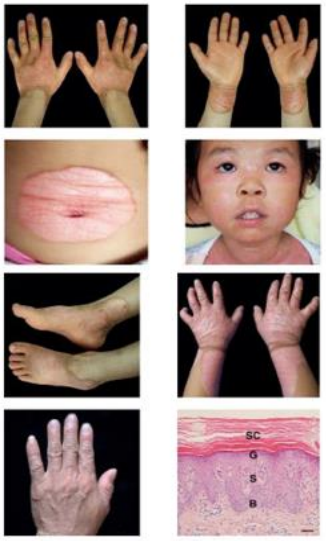

Figure 1. Clinical manifestations and histological findings of PSEK-affected individuals[1].

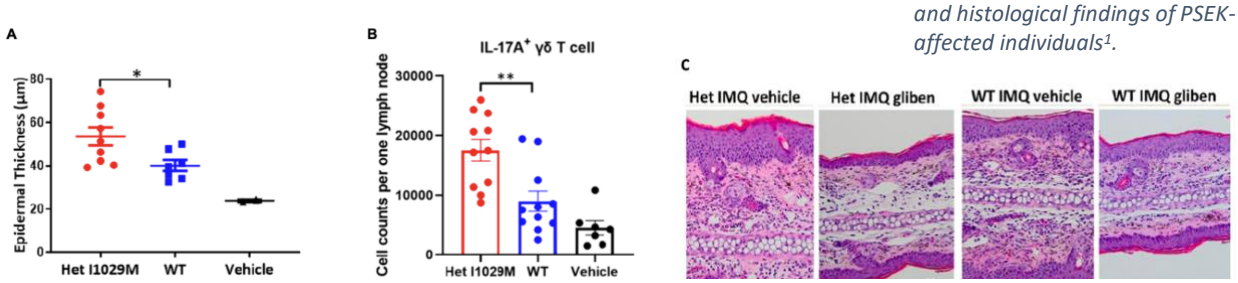

Figure 2. Het GoF TRPM4 mice showed increased epidermal thickness (A), greater numbers of IL-17A+ gd T cells in LN following IMQ treatment (B), and diminished skin inflammation in glibenclamide-treated Het GoF TRPM4 mutant and WT mice following IMQ treatment (C)[3].

to be positively correlated with psoriasis-like inflammation, and particularly enhanced when treated with IMQ. Results have indicated that elevated TRPM4 activities boost susceptibility to cutaneous stimuli, likely through elevation of membrane potential and alteration of downstream cellular signaling, resulting in enhanced inflammation. We have also shown that glibenclamide, a diabetes drug and low potency inhibitor of TRPM4, can reduce PsD in the IMQ murine skin model[3] (Fig. 2C), raising the possibility that specific targeting of TRPM4 with higher potency TRPM4 antagonists, such as compound 5[2], may provide a novel new therapeutic approach to psoriasis.

The Hwang lab has also shown that exposure to a high-sugar and fat diet in mice induces skin inflammation and enhances susceptibility to imiquimod-induced PsD[4]. Utilizing an IL-23 minicircle-based model with features of both PsD and psoriatic arthritis showed that intake of Western Diet (WD) for 10 weeks predisposed mice not only to skin but also to joint inflammation. WD-induced skin and joint pathology was associated with an expansion of IL-17A–producing $\gamma\delta$ T cells and increased expression of T helper type 17 cytokines. Because WD causes significantly more skin and joint inflammation in IL-23MC treated mice, we will analyze Compound 5's higher specificity (and lower ED50) for the TRPM4 receptor for its ability to block inflammation[2]. Since TRPM4 has been reported to regulate dendritic cell migration, we performed an in vivo DC migration experiment and found that GoF TRPM4 mutant mice showed enhanced trafficking of dendritic cells from the skin[3]. Thus, we will determine if DC proliferation and migration will be inhibited via the use of compound 5 (in vitro), and if application of compound 5 in vivo will treat the WD-promoted, IL23-mediated psoriasis in mice.

Experimental Plan and Methods:

#1: Objectives - (a) to determine if TRPM4 plays a critical role in migration of skin dendritic cells in response to chemotactic cytokines, and (b) to determine if keratinocyte proliferation is blocked by a potent TRPM4 inhibitor by in vitro culturing primary cells from TRPM4 mutant and wild-type mice

A chemotaxis assay, which is traditionally used to conduct analysis of cell migration, will be employed to assess Migratory DC characteristics through the following steps: Skin-migrating DCs will be isolated from C57BL6 mouse ears as described[5]. The cartilage-free dorsal halves will be floated dermal side down on 2ml complete medium in 24-well plates (one ear/well) and cultured for 3 days at 37℃. and centrifuged at 4℃. After removal of the skin explants, the nonadherent migratory cells will be resuspended, filtered through a 70-um filter, and centrifuged at 450×g for 5 min at 4℃. The cells will be resuspended in 100 ul in the hemocytometer using Trypan blue for counting.

For the chemotaxis assay, collected DCs will be labeled with the intracellular fluorescent dye (Q2), calcein-AM and placed with and without chemokines (i.e. CCL21). A total of 25,000 cells in 25 ul of buffer A (HBSS, 0.1% BSA) will be loaded on the top of the filter (ChemoTx 5um, Neuroprobe). After 3 hours at 37°C in a 5% CO2 incubator, the cells that accumulated at the bottom of the chamber (30ul) will be collected and added to 250ul Facs buffer for flow cytometry counting. Alternatives and Pitfalls: If sufficient numbers of skin DCs cannot be collected, bone-marrow derived DC will be harvested from mice as described[6].

Figure 1. ChemoTx, 5um, Neuroprobe for chemotaxis assay

For keratinocyte proliferation studies, HaCat cells and human primary keratinocytes will be cultured in standard culture conditions at 37 deg. C. Identical wells of keratinocyte cells will be exposed to Compound 5, glibenclamide, as well as vehicle (negative control) for 24 and 48 hours with dosing of drug agents at 0.5, 1, 5, and 10 micromolar concentrations. G, S and M phase analysis as well as cell proliferation will be measured by flow cytometry and WST-1 assay[3].

#2: Objectives - (a) to determine if inhibition of TRPM4 function will improve inflammatory symptoms in a mouse psoriasis model; (b) and if immune cells (DC, macrophage, or T cell) are regulated by compound 5 in the mice.
For in vivo dendritic cell migration assay, we will apply FITC on mice ears and evaluate the numbers of FITC+ CD11c+ cells in the draining lymph node in WT and TRPM4 GoF mutant mice. TRPM4 GoF mutant mice show increased DC migration compared to WT in this assay[3]. In proposed experiments, we will determine if compound 5 treatment (given IP at various dosing schedules and amounts) can block the increased DC migration that was previously observed. Quantification of migration will be assessed by counting the number of FITC+ CD11c+ cells in the draining lymph nodes of mice by flow cytometry[1].

To determine if Compound 5 can block psoriasis-like dermatitis in vivo, we will use the IMQ dermatitis model as described with glibenclamide[3]. We will also determine if Compound 5 can ameliorate psoriasis symptoms in mouse models beyond the IMQ model, which has known limitations[7]. We have used the IL-23 minicircle DNA hydrodynamic injection model of PsD[8,9]. Once effective dosing of Compound 5 is identified in the IMQ model, we will test them in the IL-23MC and WD models to obtain generalizable conclusions about the therapeutic effects of TRPM4 inhibitor on psoriasis models.

We will use RT-PCR to semi-quantitatively compare TRPM4 gene expression in IL-23MC-induced skin to determine if TRPM4 remains expressed in inflamed skin of mice compared to sham-treated mice.

We will determine the optimal dose for ameliorating the symptoms in IMQ-PsD with Compound 5 and will then attempt to ameliorate symptoms in both IL-23 and WD induced PsD. We will treat mice with variable doses by intraperitoneal injections of Compound 5 daily and measure ear thickness, epidermal thickness, PSI, infiltration of DCs, neutrophils and CCR6+ γδ-low T cells, and IL17a expression. We will generate a dose-response curve based on PSI and ear thickness. When the optimal dose is determined, cytokine expression profiling will be performed, including Il17f, Il12, Il23, CXCL1, TNF, and K16. IHC for p-STAT3 will be done.

Once optimal dosing is found for Compound 5, systemic toxicity be assessed with analysis of CBC and liver histology as well as measurements of serum AST and ALT.

Lesional mRNA expression will be quantified using RT-PCR methods. Protein expression will be quantified using ELISA and Western blot. Histopathology will be performed using formaldehyde-fixed, paraffin-embedded skin samples stained with H&E.

Student's t-test and ANOVA be used for comparisons between 2 treatment groups and multiple groups, respectively. Multiple test correction for 2 groups or multiple groups will be performed with Tukey's test or with the Benjamini-Hochberg method. Data analysis will be performed using R or GraphPad Prism.
Pitfalls and alternatives: Recognizing that IMQ-induced PsD is an imperfect model of human psoriasis, we have specifically included the IL-23 minicircle DNA-induced models for testing of Compound 5 to increase generalizability.

Significance for Psoriatic Disease:
Keratinocyte hyperproliferation is a key feature of psoriasis and yet poorly understood from a mechanistic point of view. Our prior work suggests that gene abnormalities in TRPM4 can trigger PSEK, which mimics some clinical features of psoriasis, including hyperproliferation. We seek to (a) understand how TRPM4 regulates psoriatic inflammation and to (b) determine if TRPM4 inhibitors can reduce psoriatic inflammation, allowing us to target new pathways that will allow us to identify improved treatment of psoriatic diseases. Thus, TRPM4 inhibitors can potentially offer a novel approach to treating psoriasis by blocking a key regulator of keratinocyte proliferation.

References

1. Wang H, Xu Z, Lee BH, Vu S, Hu L, Lee M, Bu D, Cao X, Hwang S, Yang Y, Zheng J, Lin Z. Gain-of-Function Mutations in TRPM4 Activation Gate Cause Progressive Symmetric Erythrokeratodermia. J Invest Dermatol. 2019 May;139(5):1089-1097. doi: 10.1016/j.jid.2018.10.044

2. Ozhathil LC, Delalande C, Bianchi B, Nemeth G, Kappel S, Thomet U, Ross- Kaschitza D, Simonin C, Rubin M, Gertsch J, Lochner M, Peinelt C, Reymond JL, Abriel H. Identification of potent and selective small molecule inhibitors of the cation channel TRPM4. Br J Pharmacol. 2018 Jun;175(12):2504-2519. doi: 10.1111/bph.14220

3. Yamada D, Vu S, Wu X, Shi Z, Morris D, Bloomstein JD, Huynh M, Zheng J and Hwang ST (2022) Gain-of-Function of TRPM4 predisposes mice to psoriasiform dermatitis. Front. Immunol. 13:1025499. doi: 10.3389/fimmu.2022.1025499

4. Shi Z, Wu X, Santos Rocha C, Rolston M, Garcia-Melchor E, Huynh M, Nguyen M, Law T, Haas KN, Yamada D, Millar NL, Wan YY, Dandekar S, Hwang ST. Short-Term Western Diet Intake Promotes IL-23–Mediated Skin and Joint Inflammation Accompanied by Changes to the Gut Microbiota in Mice. J Invest Dermatol. 2021 Jul;141(7):1780-1791. doi: 10.1016/j.jid.2020.11.032

5. Saeki, H., et al., Cutting edge: secondary lymphoid-tissue chemokine (SLC) and CC chemokine receptor 7 (CCR7) participate in the emigration pathway of mature dendritic cells from the skin to regional lymph nodes. Journal of immunology (Baltimore, Md.: 1950), 1999. 162(5): p. 2472-2475.

6. Wu, M. and S.T. Hwang, CXCR5-transduced bone marrow-derived dendritic cells traffic to B cell zones of lymph nodes and modify antigen-specific immune responses. J. Immunol., 2002. 168: p. 5096-5102.

7. Hawkes, J.E., J.E. Gudjonsson, and N.L. Ward, The Snowballing Literature on Imiquimod Induced Skin Inflammation in Mice: A Critical Appraisal. J Invest Dermatol, 2017. 137(3): p. 546-549.

8. Shi, Z., et al., Differential Requirement for CCR6 in IL-23-Mediated Skin and Joint Inflammation. J Invest Dermatol, 2020.

9. Shi, Z., et al., Targeting the CCR6/CCL20 axis in entheseal and cutaneous inflammation. Arthritis Rheumatol, 2021. 73: p. 2271-83.

Budget Justification:

Personnel:

Sam T. Hwang, MD, PhD, (Mentor, 0 Calendar Months)

Dr. Hwang, Professor and Chairman in the Department of Dermatology at UC Davis Medical Center, is a board-certified dermatologist with extensive training as a physician scientist. He has a strong interest in immunological diseases of the skin, including psoriasis. Dr. Hwang has 20 years of experience in the field of leukocyte and dendritic cell trafficking and has a longstanding interest in the role of chemokine receptors, including CCR6, in T cell and other immune cell trafficking to skin. Dr. Hwang has published in the J. Clinical Investigation, J. Experimental Medicine, J. Immunology, and Journal of Investigative Dermatology. He and his collaborators have published two articles on TRPM4 in the JID in 2019 and Frontiers in Immunology (2022). Dr. Hwang will provide expertise in planning the experiments outlined in the proposal.

Omar Alzayat, B.A. 2nd year medical student at UC Davis School of Medicine

Mr. Alzayat is an outstanding medical student at UC Davis School of Medicine who will be taking a year off for research in the laboratory of Dr. Sam Hwang, MD, PhD, Chair and Professor of Dermatology at UC Davis. Mr. Alzayat has already started performing research on this project since December 2022. He will be responsible for nearly all aspects of the research including both in vitro and in vivo experiments as outlined in the research proposal. He will be assisted by Dr. Xuesong Wu, MD, PhD, Senior project scientist in the laboratory of Dr. Hwang who has over 15 years of experience working on animal models of cancer and psoriasis.

Total Costs:

a. Medical Student Trainee Fellow: $8000 annual stipend
b. Travel: $2000 for Mr. Alzayat to present work from this project at the Society for Investigative Annual Meeting in 2024
c. Publication: $2000 to publish work coming from this project in the JID or equivalent high impact scientific journal
d. Mouse and housing costs: $8000 for 1 year-Approximately 100 mice at $80 per mouse
e. Flow cytometry and other reagents: $5000

Total Costs: $25,000

Indirects not included per sponsor guidelines.

Budget Table:

Start Date:	7/1/23	*Non-NIH*	**Title:**	TRPM4 Function in Western Diet Induced Psoriasis via IL-23 Mediated Inflammation	
End Date:	6/30/24		**PI(s):**	Alzayat, Omar	**12 Months**

PERSONNEL										***Salary Basis***	*	**Period 1**
Name/Role:	**Annual Salary**			**Project Period % Effort**						**Salary Basis and Type**	**Escal**	**7/1/23-6/30/24**
	Base	**Summer**	**Total**	**Per 1**	**Per 2**	**Per 3**	**Per4**	**Per5**				
1 Alzayat, Omar - Stipend			-							CAL 12/12		8,000
2			-							CAL 12/12		0
Total Salaries												**8,000**
		FY Split:	***12/0***	***0/0***		***0/0***		***0/0***	***0/0***			

TRAVEL	**International?**	**Period 1**
Travel expenses	No	2,000
	No	
	No	
Total Domestic Travel		***2,000***
Total International Travel		***0***
Total Travel		**2,000**

OTHER DIRECT COSTS		
Publication Costs		
Publication expenses		2,000
Total Publication Costs		**2,000**
Other Expenses	**Subject to IDC Calc (MTDC)?**	
Mouse expenses	Yes	8,000
Flow expenses	Yes	5,000
	Yes	
Other Expenses Subject to Indirect:		***13,000***
Other Expenses Excluded from Indirect:		***0***
Total Other Direct Costs		**15,000**

Total Direct Costs	**Choose Rate Type from Dropdown Below:**				**25,000**
Indirect Cost Base	**Rate Type:**	Other: (Enter Info Below)			25,000
Indirect Costs	**Base Type:**	**MTDC**	**Rate (%):**	0.0%	*0*
Total Costs (Direct + Indirect)					**$25,000**

Omar Alzayat

CONTACT INFORMATION:

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Sacramento, CA 95819

Mobile No: (510) 423-2043
Email No: oalzayat@ucdavis.edu

EDUCATION:

2021 – Present **Medical Student**
University of California Davis School of Medicine
Sacramento, CA
M.D. expected 2025

2017 – 2019 **Postbaccalaureate Premedical Student**
Columbia University
New York, NY

2009 – 2013 **B.A. in Economics**
University of California, Berkeley
Berkeley, CA

RESEARCH EXPERIENCE:

Researcher in Dr. Samuel Hwang's Lab
UC Davis, Department of Dermatology
Mentor: Samuel Hwang, M.D., Ph.D.
2022 – Present

Current Projects:

1.TRMP4 Function in Western Diet Induced Psoriasis via IL-23 Mediated Inflammation

This study seeks to investigate the role of a transient receptor potential cation channel (TRPM4) in the development of progressive symmetrical erythrokeratoderma and psoriasis, as well as potential therapeutics.

Specifically, we will determine if TRPM4 plays a critical role in migration of skin dendritic cells in response to chemotactic cytokines and determine if keratinocyte proliferation is blocked by a potent TRPM4 inhibitor by in vitro culturing primary cells from TRPM4 mutant and wild-type mice.

We will also determine if inhibition of TRPM4 function will improve inflammatory symptoms in a mouse psoriasis model and if immune cells (DC, macrophage, or T cell) are regulated by a TRPM4 inhibitor in mice.

Researcher in Dr. Da Zhi Liu's Lab
UC Davis, Department of Neurology
Advisor: Da Zhi Liu, Ph.D.
2022 – 2023

Completed Projects:

1. Multi-Targeted Anti-Inflammatory Drugs for the Treatment of Neurological Disorders

This research publication and relevant research leverages cancer elements, such as oncogenes/kinases (e.g. Src, Rock) and tumor suppressors (e.g. miR-122, miR-125b), to develop small molecule and microRNA drugs for treatment of traumatic brain injury, ischemic and hemorrhagic stroke, and other neurological disorders.

2. FDA-Approved Kinase Inhibitors in Preclinical and Clinical Trials for Neurological Disorders

This review publication summarizes the 74 FDA-approved kinase-targeted drugs and highlights those have been reported in preclinical and/or clinical trials for neurological disorders, with a focus on discussing feasibility and applicability of leveraging these cancer drugs for neurological treatments.

Ongoing Projects:

1.Tumor Suppressor MicroRNAs in Neurological Treatments

This review publication will provide an overview of the potential applications of miRNAs as tumor suppressors, biomarkers, and therapeutics.

Medical Student Researcher
UC Davis, Department of Internal Medicine
Advisor: Michael Wilkes, M.D.
2022 – 2023

Ongoing Projects:

1. Perceptions of vaccines amongst the patient populations of UC Davis Medical School Affiliated Student Run Clinics

This oral survey study aims to investigate the perceptions and beliefs of vaccines held by patients within UC Davis School of Medicine affiliated Student Run Clinics (SRCs). We are interested in understanding barriers and factors that influence whether or not patients within these SRC populations choose to receive vaccines. Specifically, we aim to investigate the sources of vaccine information, trust of those sources, perceptions of COVID-19 and the seasonal flu virus, access to vaccines, factors influencing vaccination decisions, and perceptions of vaccine efficacy and importance.

Medical Student Researcher
UC Davis, Department of Dermatology
Advisor: Daniel B. Eisen, M.D.
2022 – 2023

Completed Projects:

1. Patient-Provider Communication Characteristics After Mohs Micrographic Surgery

This observational study assesses the medical concerns that patients indicate through multiple modalities of communication. Through the analysis and comparison of patient data from MyChart messages and telephone calls, post-surgical instances of communication initiated by patients and the reasons that motivated patients to initiate communication are examined.

Medical Student Researcher
UC Davis, Department of Anesthesiology
Advisor: Neal Fleming, M.D., Ph.D.
2022 – 2023

Completed Projects:

1. An Observational Study Comparing Intrathoracic Pressure Changes and Stroke Volume Variation with Abdominal Insufflation

This observational study compares and correlated predictive agreement between esophageal pressure and Edwards Clearsite hemodynamic measurement changes following abdominal insufflation.

PUBLICATIONS:

1. Lui A, **Alzayat O**, Do T, Perekopskiy D, Gann M, Elgokhy TS, Gao J, Liu D. Multi-targeted anti-inflammatory drugs for the treatment of neurological disorders. Neural Regen Res. 2023 Apr;18(4):805-806. doi: 10.4103/1673-5374.353489. PMID: 36204844; PMCID: PMC9700111.

2. Lui A, Vanleuven J, Perekopskiy D, Liu D, Xu D, **Alzayat O**, Elgokhy T, Do T, Gann M, Martin R, Liu DZ. FDA-Approved Kinase Inhibitors in Preclinical and Clinical Trials for Neurological Disorders. Pharmaceuticals (Basel). 2022 Dec 13;15(12):1546. doi: 10.3390/ph15121546. PMID: 36558997; PMCID: PMC9784968.

Publications in Progress:

1. Austin Lui, **Omar Alzayat**, Timothy Do, Nina Yu, Benjamin Liang, Vidur Kailash, Da Zhi Liu. Tumor Suppressor MicroRNAs in Neurological Treatments.

2. Austin Lui, **Omar Alzayat**, Timothy Do, Nina Yu, Benjamin Liang, Vidur Kailash, Da Zhi Liu. The Role of the Aberrant Cell Cycle with Respect to Kinase Inhibitors.

3. Caroline Liu, Julio Siliezar, **Omar Alzayat**, Carly Robinson, Christine Pons, Adrianna Carter, Timothy Do, Om Patel, and Dr. Michael Wilkes. Perceptions of Vaccines Amongst the Patient Populations of UCD Medical School Affiliated Student Run Clinics.

POSTER PRESENTATIONS:

UC Davis School of Medicine Research and Poster Symposium, 03/2023:

1. Patient-Provider Communication Characteristics After Mohs Micrographic Surgery
2. An Observational Study Comparing Intrathoracic Pressure Changes and Stroke Volume Variation with Abdominal Insufflation

PAST WORK EXPERIENCE:

2020 – 2021 **Administrative Assistant**
UCSF Department of Ophthalmology
San Francisco, CA

2017 – 2019 **Student Researcher, Alice! Health Promotion**
Columbia University
New York, NY

2013 – 2017 **Analyst**
The World Bank
Washington, D.C.

CURRENT EXTRACURRICULAR ACTIVITIES:

2021 – Present **Class of 2025 Treasurer**
UC Davis School of Medicine
Sacramento, CA

2021 – Present **Clinic Director and Outreach Officer**
Shifa Community Clinic
Sacramento, CA

2022 – Present **Co-Leader**
Dermatology Student Interest Group
UC Davis School of Medicine

HONORS AND AWARDS:

White Coat Investor – Top 5 Essay Winner, 2022

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Samuel Tzen-yue Hwang

eRA COMMONS USER NAME (credential, e.g., agency login): samthwang

POSITION TITLE: Professor and Chairman, University of California Davis, Department of Dermatology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Harvard College, Cambridge, MA	AB	06/1984	Biochemistry
University Basel, Basel, Switzerland	PhD	12/1989	Biochemistry
Harvard Medical School, Boston, MA	MD	06/1991	Medicine
Brigham Women's Hospital, Boston, MA	Internship	06/1992	Medicine
Univ. California San Francisco	Residency	06/1995	Dermatology
Univ. California San Francisco	Fellowship	06/1997	Immunology

A. Personal Statement

Dr. Hwang, Professor and Chair of Dermatology at UC Davis School of Medicine, is a board-certified dermatologist with extensive training as a physician scientist. He has a strong interest in immunological diseases of the skin, including psoriasis, and in the biology of skin cancers, including melanoma and cutaneous T cell lymphoma. Starting as a scientist and then senior scientist at NIH, Dr. Hwang has had over 20 years of experience in the field of leukocyte and cancer cell trafficking and has had a long-standing interest in the role of chemokine receptors, including CCR6 and CXCR4 in psoriasis as well as cancer cells. Prior to being appointed tenured Chair and Professor of Dermatology at the University of California Davis in 2016, he was Chair and Thomas Russell Professor of Dermatology at the Medical College of Wisconsin. He has published original and review articles in the *Journal of Clinical Investigation*, *Journal of Experimental Medicine, Cancer Research, Journal of Immunology, Journal of Investigative Dermatology*, *JAMA Dermatology*, and *PNAS*. Lecturing nationally and internationally on psoriasis, Dr. Hwang is recognized for his impactful contributions to basic and translational psoriasis research.

He and colleagues at the Medical College of Wisconsin and NIH were among the first to conclusively demonstrate the role of CCR6 in the development of psoriasiform dermatitis (analogous to human psoriasis). Dr. Hwang's team has earned an international reputation in the use of murine models of psoriasis and CTCL for exploration of disease pathogenesis and treatment. He and collaborators have published extensively on the role CCL20 and CCR6 in psoriasiform dermatitis. He team proved, for example, that CCR6 was critical for the trafficking of epidermal gamma-delta T cells to the skin as Th17 effectors in murine models of psoriasis. Dr. Hwang has collaborated extensively with Dr. Brian Volkman (MCW) to develop specific chemokine receptor antagonists, including the development of a novel CCL20 locked dimeric molecule (CCL20LD) with biased agonist properties, including the inhibition of CCR6-mediated T cell migration and prevention of IL-23 mediated skin and entheseal inflammation in mice (Getschman et al. PNAS 2017; Shi et al. Arthritis & Rheumatol. 2021).

Lecturing nationally and internationally on psoriasis, Dr. Hwang is recognized for his impactful contributions to basic and translational psoriasis research. He was fortunate to have received two Discovery and two

Translational Research awards from the National Psoriasis Foundation as well as an RO1 and SBIR phase 1 and 2 support for his work on the role of CCR6 in psoriasis. In 2020, he was awarded the Psoriasis Research Achievement Award by the American Skin Association.

Dr. Hwang is most grateful for past support from GRAPPA. While PSEK is a rare disease, the phenotypic similarity to psoriasis is striking, and our preliminary results, now published in Yamada et al. Frontiers in Immunology 2022, suggest that TRPM4 may indeed play a role in psoriasiform dermatitis, thus offering a new therapeutic target for improved treatment of psoriasis patients. I am excited to apply the techniques we have learned with respect to animal models of psoriasis to test this hypothesis as outlined in Mr. Omar Alzayat's grant proposal.

Ongoing and recently completed projects that I would like to highlight include:

National Psoriasis Foundation
Bridge Grant Awarded to Scott I. Simon, PhD 2023-2024
Neutrophil priming in the circulation amplifies effector function that exacerbates psoriasis
Role: Co-PI

University of California School of Medicine
Pilot Awards for Innovative Studies of a Whole-body, High Resolution Positron Emission Tomography/CT device 2022-2023
PET EXPLORER as a tool for measuring systemic inflammatory changes following short-term dietary intervention
Role: PI

National Psoriasis Foundation
Translational Research Award 2020-2022
Dietary determinants of susceptibility to IL-23-mediated, psoriasis-like skin and joint inflammation
Role: PI

National Psoriasis Foundation
Bridge Award 2021-2022
CCR6 in psoriatic skin and joint disease
Role: PI

NIAMS – SBIR (Phase I)
1R43AR074363-01 2018-2019
Development of an engineered CCL20 protein as a lead therapeutic molecule for psoriasis
Role: Associate PI (PI: Anthony Getschman)

NIAMS- SBIR (Phase II)
2R44AR0743363-02 2020-2022
Development of an engineered CCL20 protein as a lead therapeutic molecule for psoriasis
Role: Associate PI (PI: Anthony Getschman)

Pfizer
Pfizer Aspire Award 2018-2020
CCR6 as a target for IL-23 mediated skin and joint inflammation
Role: PI

Citations:

Getschman AE, Imai Y, Larsen O, Peterson FC, Wu X, Rosenkilde MM, **Hwang ST**, Volkman BF. Protein engineering of the chemokine CCL20 prevents psoriasiform dermatitis in an IL-23-dependent murine model. *Proc Natl Acad Sci U S A*, 114(47): 12460-12465. 2017.

Shi Z, Wu X, Yu S, Huynh M, Jena PK, Nguyen M, Wan YY, **Hwang ST**. Short-Term Exposure to a Western Diet Induces Psoriasiform Dermatitis by Promoting Accumulation of IL-17A-Producing γδ T Cells. *Journal of Investigative Dermatology*, 140(9): 1815-1823. 2020.

Shi, Z. Wu, X. Rocha, C. S. Rolston, M. Garcia-Melchor, E. Huynh, M. Nguyen, M. Law, T. Haas, K. N. Yamada, D. Millar, N. L. Yvonne Wan, Y. J. Dandekar, S. **Hwang, ST**. Short-term Western diet intake promotes IL-23-mediated skin and joint inflammation accompanied by changes to the gut microbiota in mice. Journal Invest. Dermatol., 141(7): 1780-91. 2021.

Shi, Z. Garcia-Melchor, E. Wu, X. Getschman, A. E. Nguyen, M. Rowland, D. J. Wilson, M. Sunzini, F. Akbar, M. Huynh, M. Law, T. Kundu-Raychaudhuri, S. K. Raychaudhuri, S. P. Volkman, B. F. Millar, N. L. **Hwang, S. T.** Targeting the CCR6/CCL20 axis in entheseal and cutaneous inflammation. Arthritis & Rheumatology, E-publication. 2021.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2022-present	Section Editor, Journal of Investigative Dermatology
2019-present	Adjunct Chair Professor, Department of Dermatology, Kaohsing Medical Univ., Taiwan
2019-2021	Editorial Board Member, International J. of Dermatol. And Venereol.
2017-present	SID representative to AAD Council on Science and Research
2016-present	Chair and Professor of Dermatology, Univ. California Davis School of Medicine
2016-2021	Board of Directors, Society for Investigative Dermatology
2013-2018	Board of Directors, US Cutaneous Lymphoma Consortium
2012-2017	Section Editor, Journal of Investigative Dermatology
2008-2015	Chair and Professor of Dermatology, Medical College of Wisconsin
2007-2017	Editorial Board, Journal of Dermatol. Science (Japan)
2007-present	Member American Society for Clinical Investigation
2007-2012	Associate Editor, J. Investigative Dermatology
2004-2008	Senior Investigator, Dermatology Branch, National Cancer Institute
2002-2008	Advances in Dermatology, Editorial Board
1997-2004	Investigator, Dermatology Branch, National Cancer Institute
1994-1996	Howard Hughes Physician Fellow, University of California San Francisco

Honors

2022	Best Doctors (Dermatology) Sacramento Magazine
2022	Larry Vanderhoef Memorial Lecture: National Chung-hsing University, Taichung, Taiwan
2022	Keynote Speaker: National Psoriasis Foundation Dermatology Resident Educational Retreat
2021	Keynote Speaker, Taiwanese Society for Investigative Dermatology, Taipei, Taiwan
2021	Keynote Speaker, Frontiers in Immunology Virtual Meeting, Changsha, China
2021	Best Doctors (Dermatology) Sacramento Magazine
2020	Best Doctors (Dermatology) Sacramento Magazine
2020	Keynote Speaker, Chinese Society for Investigative Dermatology Annual Virtual Meeting Xian China
2020	American Skin Association Award for Research in Psoriasis
2019	Best Doctors (Dermatology) Sacramento Magazine
2019	Keynote Speaker, Taiwanese Society for Investigative Dermatology Annual Meeting Kaohsiung
2019	Plenary Speaker, Chinese Society for Investigative Dermatology Annual Meeting, Changsha
2018	Best Doctors (Dermatology) Sacramento Magazine
2017	Keynote Speaker, Taiwanese Society for Investigative Dermatology Annual Meeting Taipei
2016	Plenary Speaker, Japanese Dermatological Assoc., Central Division, Annual Meeting
2014	Plenary Speaker, JID Shanghai International Dermatology Workshop, Shanghai
2014	10th Anniversary Advancing a Healthier Wisconsin Collaborative Science Award
2014	Plenary Speaker, Tokai University Psoriasis Research Meeting, Tokyo, Japan
2013	Plenary Speaker, Korean Society for Investigative Dermatology, Seoul

2013 Plenary Speaker, Taiwanese Society for Investigative Dermatology, Taipei
2011 Hubert Moss Lecture, Univ. Wisconsin-Madison, Dept. of Dermatology
2011 Visiting Professor, Univ. of Minnesota, Dept. of Dermatology
2010 Keynote Lecture, Milwaukee Academy of Medicine, Milwaukee, WI
2009 Keynote Speaker, Japanese Society for Psoriasis Research, Tokyo
2007 Membership, American Society for Clinical Investigation (ASCI)
2006 International Academic Dermatologist Recognition (Western Japanese Journal of Dermatology)
2004 Astellas/Yale Department of Dermatology Lectureship
1999 Keynote Lecture, University of Tokyo Annual Dermatology Meeting
1998 Albert Kligman Fellowship, International Invest. Dermatol, Cologne, Germany
1996 American Academy of Dermatology Young Investigator Award

C. Contributions to Science

Role of chemotactic receptors in immune cell trafficking and cancer metastasis

Dr. Hwang's initial scientific work focused on the elucidation of the role of chemokine receptors in trafficking of leukocytes and cancers to inflammatory tissues (skin) and sites of metastasis, respectively (T Kakinuma and ST Hwang. 2006).

Among the first to explore the role of chemokine receptors in antigen-presenting cell trafficking, his early immune biology work focused on the migratory chemotactic trafficking of dendritic cells from the skin to regional lymph nodes via CCR7 (Saeki H et al J. Immunol.1999).

In paradigm shifting later work, he showed that melanoma cells could express CCR7 and used this receptor to facilitate nodal metastasis, one of the major negative prognostic factors in melanoma as well as many other solid tumors (HE Wiley, et al. J. Natl. Cancer Inst., 2001). This work was among the first to demonstrate that cancer cells could usurp physiologic mechanisms of cellular trafficking, resulting in metastasis to distant sites.

A. Kakinuma, T, **Hwang, ST**. Chemokines, chemokine receptors, and cancer metastasis. *Journal of leukocyte biology*, 79(4): 639-51. 2006.
B. Saeki, H, AM Moore, MJ Brown, and **ST Hwang**. Cutting Edge: Secondary Lymphoid-tissue Chemokine (SLC) and CCR7 participate in the emigration pathway of mature dendritic cells from the skin to regional lymph nodes. *J. Immunol.*, 162: 2472-5. 1999.
C. HE Wiley, III, EB Gonzalez, W Maki, M Wu, **ST Hwang**. Expression of CC chemokine receptor-7 (CCR7) enhances regional lymph node metastasis of B16 murine melanoma. *J Natl. Cancer Inst.*, 93: 1638-43. 2001.

Investigation of the role of CCR6 in psoriasis

Since 2009, his interests have shifted toward understanding the pathogenesis of psoriasis using murine models of this disease. His laboratory was the first to describe the critical role of CCR6 in trafficking of gamma-delta T cells to the epidermis in psoriasis (MN Hedrick et al. J. Clin. Invest. 2009; Mabuchi T et al. J Immunol. 2011). This body of work demonstrated that CCR6 was vital in order for T helper 17 cells that heavily express CCR6 to migrate to skin and to the epidermis where they produced IL-22 and IL-17, major drivers of psoriatic inflammation. He is considered an expert in the use of IL-23 and imiquimod murine models of psoriasis.

Dr. Hwang and Dr. Volkman collaborated to show that an engineered, dimeric CCL20 molecule (called CCL20LD) could block IL-23-mediated psoriasiform dermatitis (Getchman et al. PNAS 2017). Most recently, in collaboration with Dr. Volkman, Millar, and Raychaudhuri, Dr. Hwang and colleagues showed that CCL20LD could block IL-23-mediated joint inflammation in mice, raising the possibility that blockade of CCR6 could be a novel therapeutic pathway in psoriatic arthritis (Shi et al. 2021)

A. MN Hedrick, A Lonsdorf, A Shirakawa, **ST Hwang**, and JM Farber. CC chemokine receptor-6 (CCR6) is essential for IL-23-mediated psoriasiform dermatitis in mice. *J. Clin. Invest.*, 119: 2317-29. 2009.

B. Mabuchi T, Takekoshi T, **Hwang ST**. Epidermal CCR6+ γδ T cells are major producers of IL-22 and IL-17 in a murine model of psoriasiform dermatitis. *J Immunol*, Nov 15;187(10): 5026-31. 2011.
C. Getschman AE, Imai Y, Larsen O, Peterson FC, Wu X, Rosenkilde MM, **Hwang ST**, Volkman BF. Protein engineering of the chemokine CCL20 prevents psoriasiform dermatitis in an IL-23-dependent murine model. *Proc Natl Acad Sci U S A*, 114(47): 12460-12465. 2017.
D. Shi, Z. Garcia-Melchor, E. Wu, X. Getschman, A. E. Nguyen, M. Rowland, D. J. Wilson, M. Sunzini, F. Akbar, M. Huynh, M. Law, T. Kundu-Raychaudhuri, S. K. Raychaudhuri, S. P. Volkman, B. F. Millar, N. L. **Hwang, S. T.** Targeting the CCR6/CCL20 axis in entheseal and cutaneous inflammation. *Arthritis & Rheumatology*, E-publication. 2021.

Role of Western Diet in increasing susceptibility of skin to psoriasiform inflammation

Since 2018, Dr. Hwang has been collaborating with Dr. Yvonne Wan, a respected NIH/NCI funded investigator at UC Davis and an expert in the metabolic and neoplastic changes in the liver that are mediated by the so-called Western diet (WD) comprised of moderate-to-high fat and high sucrose content. After applying this diet to mice, we have published both long-term changes to the skin (Jena et al. J. Dermatol. Sci. 2019), leading to increased susceptibility to imiquimod-mediated dermatitis (Yu et al. J. Invest Dermatol. 2019). In our most recent reports, we show that short-term (4 weeks) feeding of the WD in standard laboratory mice results in clinical signs of psoriasiform skin inflammation that was dependent on CCR6+ gamma-delta T cells and accompanied by up-regulation of Th17 cytokines characteristic for human psoriasis (Shi et al J. Invest. Dermatol. 2020). Subsequent work demonstrated remarkable changes in the gut microbiome from mice that had been subjected to the WD and systemic IL-23 treatment (Shi et al. J. Invest. Dermatol. 2021).

A. P. Jena, L. Sheng, K. Mcneil, T.Q. Chau, S.Yu, M. Kiuru, M.A. Fung, **S.T. Hwang**, Y.J. Wan. Long-term Western diet intake leads to dysregulated bile acid signaling and dermatitis with Th2 and Th17 pathway features in mice. *Journal of Dermatological Sciences*, 95(1): 13-20. 2019.
B. Yu S, Wu X, Zhou Y, Sheng L, Jena PK, Han D, Yvonne Wan YJ, **Hwang ST**. A Western Diet, but Not a High-Fat and Low-Sugar Diet, Predisposes Mice to Enhanced Susceptibility to Imiquimod-Induced Psoriasiform Dermatitis. *Journal of Investigative Dermatology*, 139: 1404-1407. 2018.
C. Shi Z, Wu X, Yu S, Huynh M, Jena PK, Nguyen M, Wan YY, **Hwang ST**. Short-Term Exposure to a Western Diet Induces Psoriasiform Dermatitis by Promoting Accumulation of IL-17A-Producing γδ T Cells. *Journal of Investigative Dermatology*, 140(9): 1815-1823. 2020.
D. Shi, Z. Wu, X. Rocha, C. S. Rolston, M. Garcia-Melchor, E. Huynh, M. Nguyen, M. Law, T. Haas, K. N. Yamada, D. Millar, N. L. Yvonne Wan, Y. J. Dandekar, S. **Hwang, S. T.** Short-term Western diet intake promotes IL-23-mediated skin and joint inflammation accompanied by changes to the gut microbiota in mice. *Journal Invest. Dermatol.*, 141(7): 1780-91. 2021.

Elucidation of the pathogenesis of cutaneous T cell lymphoma

Dr. Hwang has also made significant strides in improving our understanding of the pathophysiology of cutaneous T cell lymphoma, a rare form of non-Hodgkin's T cell lymphoma that primarily affects the skin. Because of the lack of spontaneous mouse model of CTCL, Dr. Hwang's lab has developed a novel animal model in which to study the formation of T cell lymphoma in the skin microenvironment (Wu X et al. J Invest Dermatol. 2011). Importantly, this model is dependent on an inflammatory stimulus and responds to commonly used treatments that benefits patients with CTCL. Using this model as well as xenografted human CTCL models, he has demonstrated a role for macrophages in the development of T lymphomas in skin (Wu X et al. Depletion of M2-like tumor-associated macrophages delays cutaneous T cell lymphoma development in vivo. J Invest Dermatol. 2014) and that novel agents such as gallium maltolate (Wu X et al. J. Invest. Dermatol. 2015) or CCR2 antagonists (Wu X et al. J. Invest. Dermatol. 2020). may provide new therapeutic options for patients with CTCL

A. Wu X, Sells RE, **Hwang ST**. Upregulation of inflammatory cytokines and oncogenic signal pathways preceding tumor formation in a murine model of T-cell lymphoma in skin. *J Invest. Dermatol*, Aug;131(8): 1727-34. 2011.
B. Wu X, Schulte BC, Zhou Y, Haribhai D, Mackinnon AC, Plaza JA, Williams CB, **Hwang ST**. Depletion of M2-like tumor-associated macrophages delays cutaneous T-cell lymphoma development in vivo. *J. Invest Dermatol*, Nov;134(11): 2814-22. 2014. 2014.
C. Wu X, Wang TW, Lessmann GM, Saleh J, Liu X, Chitambar CR, **Hwang ST**. Gallium maltolate inhibits human cutaneous T-cell lymphoma tumor development in mice. *J Invest Dermatol*, Mar;135(3): 877-84. 2015.
D. Wu, X. Singh, R. Hsu, D. K. Zhou, Y. Yu, S. Han, D. Shi, Z. Huynh, M. Campbell, J. J. Hwang, S. T.. A Small Molecule CCR2 Antagonist Depletes Tumor Macrophages and Synergizes with Anti-PD-1 in a Murine Model of Cutaneous T-Cell Lymphoma (CTCL). J. Invest. Dermatol., 140: 1390-1400 e4. 2020.

Complete list of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/sam.hwang.1/bibliography/public/

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Feb. 23, 2023

Dear GRAPPA Grant Review Committee Member:

It is a pleasure for me to provide a letter of support to Mr. Omar Alzayat for his proposal related to TRPM4 as a target for therapy in psoriasis. As a physician scientist with over a decade of interest in psoriatic immunology and pathogenesis, my lab has published in the Journal of Clinical Investigation, Journal of Immunology, PNAS, and Journal of Investigative Dermatology among others.

The project Mr. Alzayat describes in his proposal was based on our recent exciting findings on the TRPM4 receptor transgenic animal that we published in Frontiers in Immunology in 2022 **(Yamada D, Vu S, Wu X, Shi Z, Morris D, Bloomstein JD, Huynh M, Zheng J, Hwang ST. Gain-of-function of TRPM4 predisposes mice to psoriasiform dermatitis. Front Immunol. 2022).** The mutation we induced in TRPM4 mimics one of two known mutations in TRPM4 that cause the skin disease known as progressive symmetric erythroderatodermia (PSEK, Wang et al. J. Invest. Dermatol. 2019), a fascinating skin disease characterized by thickened red, skin plaques that are reminiscent of human psoriasis. In our Frontiers publication, we demonstrated that TRPM4 gain-of-function mice are more susceptible to induction of psoriasis-like disease using the topical immunomodulator, imiquimod. Omar plans to step away from medical school at UC Davis School of Medicine to take a year of intensive research in my laboratory at UC Davis. In the experiments delineated in Omar's proposal, Omar will spend his research year elucidating the cellular mechanisms of the enhanced sensitivity to psoriasis-like inflammation that is observed in TRPM4 mutant mice, thus furthering our understanding of how this gene may regulate skin hyperproliferation. Moreover, we will use published unique small molecules to test the hypothesis that inhibition of TRPM4 may be a novel therapeutic approach to treating humans with psoriatic disease.

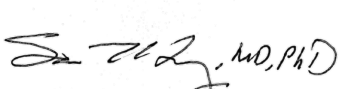
Omar approached me this summer about his desire to understand human skin disease at a basic level. He was intrigued by my lab's use of genetic animal models as well as advanced vitro cellular analytical methods (flow cytometry and migration assays) and thought our TRPM4 project would allow him to advance his laboratory research skills and increase his basic science

knowledge of skin immunology. My laboratory has multiple instruments (such as a 4 color flow cytometer, histology equipment, qPRC equipment, as well as a confocal microscope) to advance our work. We are quite adept at cell trafficking experiments as demonstrated in our Frontiers in Immunology publication. Our published work on murine models of psoriasis have been recognized in the scientific and popular press, including a recent review in Nature Reviews Rheumatology.

Omar was aware that his prior research experiences were mostly clinical in nature, but expressed a sincere desire to expand his horizons. Omar has been participating in my weekly lab meetings ever since and has started his research journey with us by diving into skin immunology research with complete commitment and excitement. It was not surprising to me that he has already started to gather interesting scientific data, even at this early juncture in my lab. I was also astonished by his ability to write a first draft of his research proposal with minimal revisions based on his careful reading of our TRPM4 publication and a few preliminary discussions. Medical students are rarely this adept at translating ideas into research plans, and I was thoroughly impressed with his effort and writing skills. I am quite pleased with the final plan and think it accurately conveys the significance of the research as well as the proposed strategy for addressing the questions that came out of our initial publication. In addition, my lab staff have been thoroughly impressed with his commitment to the lab, even though he still has a busy medical student didactic schedule as well as his ability to learn laboratory techniques.

In summary, Omar is a truly intelligent and outstanding medical student from UC Davis and plans to apply to dermatology residency after graduation. His genuine dedication to our laboratory efforts have been recognized by my lab members as well as myself. His ability to express himself and to understand fairly complicated scientific laboratory discussion demonstrates a rather unique ability to synthesize complex data that will serve him well should he receive the research grant. I highly recommend him for this grant award and look forward to working with him, regardless of the outcome, because I believe he will help us learn even more about skin pathophysiology through our unique mouse model. We look forward to having him join our laboratory on a more full-time basis in the near future. Thank you for considering his GRAPPA research project in Dermatology.

Sincerely yours,



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